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Short Communication

CO-INFECTIONS OF CLASSICAL SWINE FEVER VIRUS, PORCINE SAPELOVIRUS AND PORCINE CIRCOVIRUS 2 IN DIARRHEIC PIGLETS: EVIDENCE FROM KARNATAKA, INDIA

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ABSTRACT: Suckling and weaned piglets suffering from gastrointestinal disorders often develop diarrhea due to viral co-infections. Classical swine fever virus, porcine sapelovirus, and porcine circovirus type 2 are important pathogens that pose a threat to the swine farming industry. We analyzed 25 samples of piglet diarrhea from three different farms (A, B and C) in Karnataka for classical swine fever virus (CSFV), porcine sapelovirus (PSV) and porcine circovirus type 2 (PCV2) by RT-PCR and PCR followed by phylogenetic analysis for identifying their genotypes and sub-genotypes. Concurrent infection of PSV-PCV2 and PSV-CSFV were identified in Farm A and B, while Farm C had only PSV infection. Co-infection of these viruses resulted in varying mortality rates depending on the viral combination. The phylogenetic analysis revealed PSV1 had three distinct clusters from three different farms, Farm B CSFV genetic analysis showed 2.2 sub-genotypes, and PCV2 from Farm A showed its lineage to PCV2b type. This is the first report of PSV-PCV2 and CSFV-PSV co-infections in diarrheic piglets in Karnataka, India. Phylogenetic analysis identified distinct viral strains circulating in the region. The findings establish a correlation between co-infection patterns and mortality rates, providing valuable epidemiological insights for disease management. Additionally, the detection of CSFV from fecal samples offers a new perspective on its diagnostic approaches, highlighting the need for improved surveillance and biosecurity measures.

Keywords: Diarrheic piglets, Co-infection, CSFV, PSV, PCV2.

Diarrhea in piglets is a major economic concern in swine farming, often leading to high morbidity and mortality. Morbidity and mortality are often a multifactorial problem, involving pathogens such as bacteria, viruses and parasites. Especially when suckling and weaned piglets are affected, the primary consequences are dehydration due to diarrhea and tends to be more severe. Among the viral pathogens associated with diarrhea in piglets, classical swine fever virus (CSFV), porcine sapelovirus (PSV), and porcine circovirus type 2 (PCV2) are particular concern due to their potential to cause severe gastrointestinal disorders and immune suppression. Co-infections involving these viruses can worsen disease severity, leading to dehydration, impaired growth, and potentially death.

Classical swine fever (CSF) is a very high contagious disease of domestic pigs and wild boars, caused by *Pestivirus suis* from the family Flaviviridae. CSFV is a (+) ssRNA virus with a 12.3kb genome [1, 2], that includes non-coding regions (5'UTR and 3'UTR) and a single open reading frame (ORF) encoding structural proteins (C, ERNS, E1 and E2) as well as nonstructural proteins (Npro, P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) [3]. CAFV diagnosis is performed using ELISA, immunofluorescence, RT-PCR. RT-qPCR, and virus isolation, while its detection in fecal matter is unreported but possible, with genetic diversity studied via NS5B [4, 5, 6].

Porcine sapelovirus (PSV), a member of the Picornaviridae family, is a common cause of viral

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enteritis in piglets. PSV (*Sapelovirus anglia*), is a (+) ssRNA with a genomic length of 7.5-8.3 kb in size, genome has 5'UTR and 3'UTR with single open reading frame (ORF), the genome organization as follows, 5'UTR-L-VP4-VP2-VP3-VP1-2A-2B-2C-3A-3B-3C-3D-3'UTR. VP1-VP4 are the structural proteins and rest are the nonstructural proteins. PSV is detected using RT-PCR from fecal, rectal swab and blood samples, with antigen identification via serum, while its genetic diversity is studied using whole genome and 5'UTR [7, 8]. In pigs, porcine circovirus-associated disease (PCVAD) is mainly caused by PCV2. PCV2 (*Circovirus porcine 2*) belongs to the family Circoviridae has a ssDNA with approximate genomic length of 1.7kb in size [9, 10]. The genome contains two open reading frames, ORF1 and ORF2, which encode replication-associated non-structural proteins (Rep and Rep') and structural protein (Capsid). PCV2 is detected using ELISA and PCR from serum, tissue, and fecal samples, while genotyping and phylogenetic analysis of ORF2 reveal its sub-genotypes [11].

Limited data is available on the co-infections of CSFV, PSV, and PCV2 in the Indian swine population, despite the existence of separate investigations on these viruses. Therefore, the purpose of this work was to identify co-infection patterns, detect these viruses in diarrheal piglets, and utilize phylogenetic analysis to comprehend strain diversity and the influence of farm-level mortality.

The study

A total of 25 fecal samples were collected from piglets exhibiting diarrhea from three farms (A, B, and C) in Karnataka, India. Farms were selected due to a sudden change in climate, with an outbreak of diarrhea among piglets reported between December 2024 and January 2025. Piglets were chosen based on clinical signs, including fever, lethargy, and the severity of diarrhea. Total RNA and DNA were extracted from a 10% fecal suspension in PBS (lysate), using QIAamp Viral RNA Mini kit (Cat. No. 52904) and QIAamp DNA Mini kit (Cat. No. 51304). The prepared lysate was used as sample and followed the manufacturer's instructions for genomic isolation, after that RNA was stored at –80 ^{0}C, while DNA was stored at -20 for preservation until future analysis. One-step reverse transcriptase polymerase chain reaction was performed for both CSFV and PSV, using Takara Bio's PrimeScript One Step RT-PCR Kit Ver.2-Dye Plus (Cat. No. RR057A), while PCV2 PCR is performed using Takara Bio's EmeraldAmp GT PCR Master Mix (Cat. No. RR310A). CSFV, PSV, and PCV2 were amplified by PCR using specific oligo-nucleotide primers (Table 1). All RT-PCR and PCR was performed in 25µl reaction volume according to the manufacturer's instruction with optimal annealing temperature (Table 1). The amplified PCR products was purified with NucleoSpin Gel and PCR Clean-up (Cat. No. 740609.50) and confirmed by direct sequencing of purified product using specific primer used for amplification at Barcode Bioscience, Bangalore a sequencing facility. The Phylogenetic analysis is performed based on different genes for different viruses, CSFV phylogenetic analysis is based on partial NS5B region, PSV phylogenetic is based on conserved 5'UTR region, while PCV2 phylogeny is based on full length ORF2 region. For phylogenetic analysis, from GenBank various genotypes of different geographic region for corresponding organism's sequence were retrieved. Using MUSCLE, the sequence alignment is performed in MEGA v.11 software followed by generating a Maximum Likelihood (ML) phylogenetic tree with 1000 bootstrap replicates.

Results and discussion

In this study, piglet diarrhea samples were collected from three different farms (A, B, and C) to investigate the prevalence of coinciding infection. The results obtained from RT-PCR and PCR gave an idea about

Table 1. Primers used in this study.

Virus and Genomic region	Primer Sequence (5'-3')	Annealing temperature	Product size (bp)	Reference
CSFV				
NS5B-FP	GACACTAGYGCAGGCAAYAG	50°C	449	[5]
NS5B-RP	AGTGGGTTCCAGGARTACAT			
PSV				
5'UTR-FP	CGTGCTCCTTTGGTGATTC	53°C	270	[20]
5'UTR-RP	GAAAGAGTAGTAGTAGATTCC			
PCV2				
ORF2-FP	CCATGCCCTGAATTTCCATA	57°C	802	[21, 22]
ORF2-RP	ACAGCGCACTTCTTTCGTTT			

Table 2. Distribution of viral co-infection in piglets with diarrhoea of different farms in Karnataka, India.

Farm	Location	Total population	Symptoms	No. of deaths	Samples Tested	CSFV (+)	PSV (+)	PCV2 (+)	Co-infection	Mortality rate
A	Bengaluru	15	Fever, severe watery diarrhea, impaired growth	2	14	0/14	6/14	8/14	PSV, PCV2	13.3%
B	Mandya	110	Fever, lethargy, yellowish diarrhea	48	4	4/4	4/4	0/4	CSFV, PSV	43.63%
C	Hassan	350	Watery diarrhea	10	7	0/7	7/7	0/7	PSV	2.85%

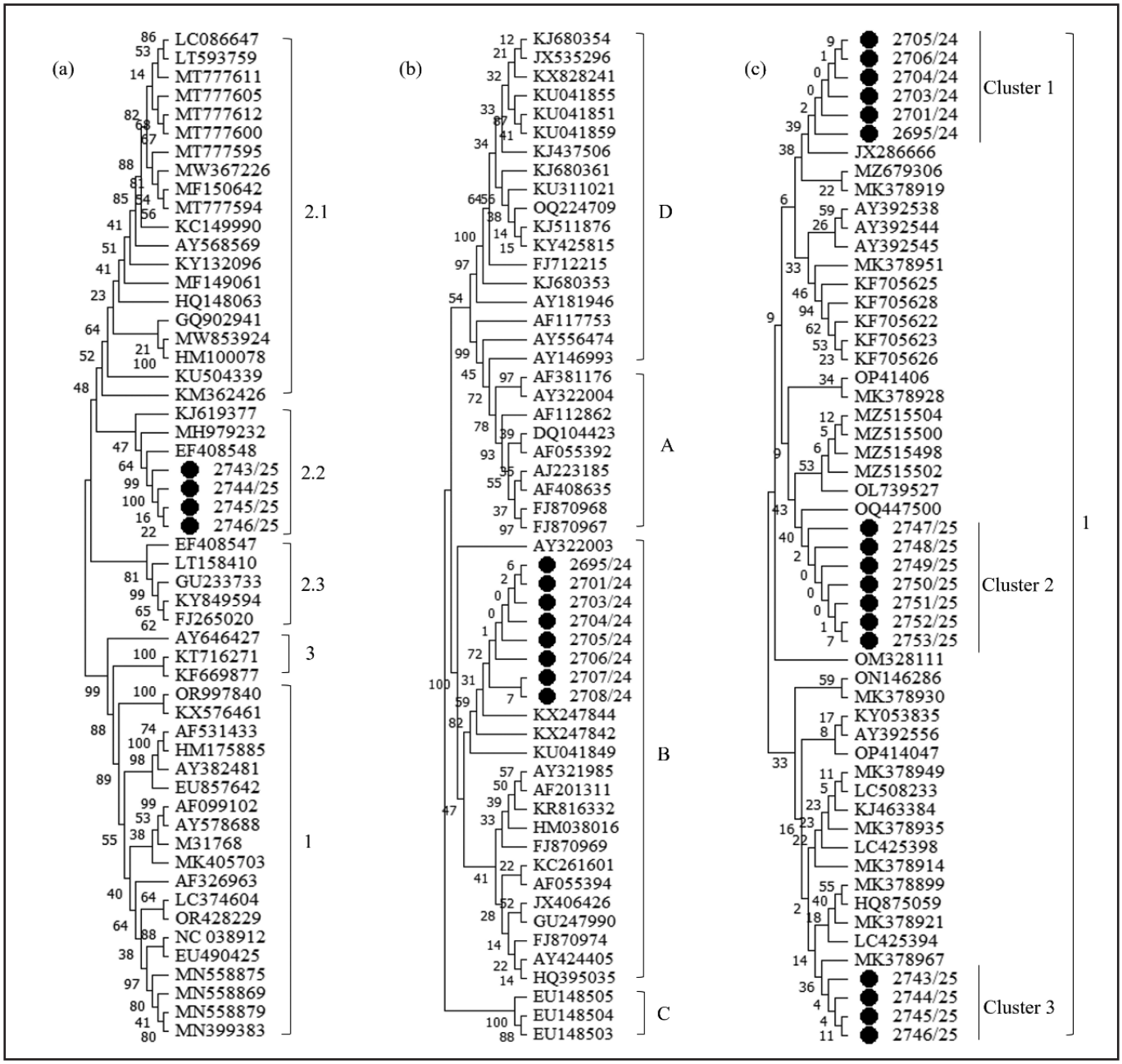

Fig. 1. Phylogenetic analysis based on the AICC model for maximum likelihood with 1000 bootstrap replicates. [(a) and (b) tree was constructed using the GTR+G+I model, while the (c) tree was constructed using K2+G+I. (a) The CSFV sequences clustered within 2.2 sub-genotype, (b) PCV2 sequence clustered into type B and (c) PSV1 revealing three distinct clusters representing different sub-genotypes].

multi-viral presence and their impact on the piglet's health. CSFV, PSV and PCV2 are the pathogens we have tested here to find their coinciding infection and the mortality rates on different farms (Table 2).

Farm A, exhibited PSV and PCV2 co-infections, with a moderate mortality rate of 13.3%. Clinical symptoms include severe watery diarrhea and impaired growth. The co-infection of PSV and PCV2 may have to contribute to critical disease manifestation compared to farm with single infection. Yet the mortality was still lesser than the farms with more complex and more intense co-infections.

Farm B, revealed the presence of both CSFV and PSV, which may have contributed to highest mortality rate being 43.63%. Piglets on this farm showed various clinical symptoms including high fever, lethargy and yellowish diarrhea, indicating that CSFV infection played a significant role in worsening disease outcomes. Concurrent infection of CSFV and PSV, enhanced the severity of the disease, leading to greater economic loss with high mortality.

Farm C, where only PSV was detected and had the lowest mortality rate being 2.85% of all the farms. Less severe disease outcome likely to be the absence of virulent pathogens such as CSFV and PCV2. The piglets only exhibited mild watery diarrhea and the clinical symptoms were less severe. In our study, co-infection of CSFV and PSV corresponded to the highest mortality (43.63%), consistent with previous reports where CSFV involvement aggravated disease outcomes [12]. In contrast, PSV-only infections showed minimal mortality (2.85%), highlighting its relatively mild pathogenic role.

The phylogenetic analysis of CSFV, PCV2 and PSV are detailed, highlighting and illustrating the genetic diversity of the viruses (Fig. 1). The PSV strains across all three farms A, B and C were belongs to PSV1 genotype within the genotype all the farms exhibited distinct sub-genotypes of PSV indicating circulation of genetically different strains. These variations could potentially influence the co-infection and pathogenicity of the virus. Although phylogenetic analysis based on the conserved 5' UTR region of PSV provides limited insights into viral variation, whole-genome analysis or VP1 sequencing may offer a more detailed understanding. On Farm A, PSV infection along with PCV2 was confirmed and subsequently the phylogenetic analysis identified the virus belongs to PCV2b. Since the mortality rate being moderate compared to others it is safe to assume PCV2b infection maybe subclinical. Poor management and biosecurity may lead to progression of subclinical infection to more severe illness. The CSFV strains from Farm B found to belong to a distinct clade group with sub-genotype 2.2, suggesting lapses in biosecurity protocol such as possible introduction of the virus by contaminated feed, contaminated equipment's or uncontrolled pig trades.

Stillbirth, Mummification, Embryonic Death, and Infertility (SMEDI) syndrome is associated with various pathogens including PCV2 and PSV. PSV is not a primary virus associated with SMEDI like PCV2, but it can still play a role in reproductive failures, particularly in co-infections or immunocompromised animals [13, 14]. Viral co-infection is more common and very much complicated due to virus-virus interaction and host impact on the viral transmission followed by changes in the pathogenicity of the virus [15]. PCV2 is well known pathogen associated with co-infection, PCV2b used to be a dominant genotype during the mid-2000s and it is closely linked with widespread of PCVAD outbreaks and genetic variation within the strains can affect vaccine efficacy [9]. Incidence of diarrheic piglet had co-infection of porcine delta-coronavirus (PDCoV) with porcine sapelovirus (PSV), in Henan provenance, China exhibited acute diarrhea leading to intestinal lesions, villi damage and fluid accumulation [16]. Co-infection of PSV with other swine viruses such as porcine teschovirus (PTV), porcine enterovirus (PEV), and porcine epdemic diarrhea virus (PEDV) had been reported frequently [17, 18]. Reports of CSFV co-infection with PCV2 and porcine reproductive and respiratory syndrome virus (PRRSV) in China during the period between 2016 and 2018 [12].

Inevitably concede that our study is limited by the relatively small size of samples (n=25), collected from three distinct farms in Karnataka affected by diarrhea outbreaks, likely due to climate changes. However, these findings provide the first report of PSV-PCV2 and CSFV-PSV co-infections in diarrheic piglets, providing insights into their impact on disease severity and farm management [19]. reported the presence of PSV in diarrheic piglets in India, our findings further emphasize the need for broader surveillance to understand the role of viral co-infections in disease outbreaks.

Conclusion

In conclusion, the observed mortality rates appeared to be influenced by the combination of viral infections present. Concurrent infection may lead to varied and potentially unpredictable outcomes. PSV infection alone showed very less mortality followed by combination of PCV2 and PSV being moderate, and the highest mortality was associated with concurrent infection

of CSF and PSV. To mitigate the economic losses associated with concurrent infection in pig herds with higher mortality rate, effective biosecurity measures implementation and timely detection of pathogens is imperative.

Ethical statement

Piglets' diarrheal samples were taken by certified veterinarians following ICAR-NIVEDI guidelines and globally recognized animal welfare standards. Informed agreement from farm stakeholders was obtained for all procedures, guaranteeing minimal discomfort and strict adherence to biosafety protocols.

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